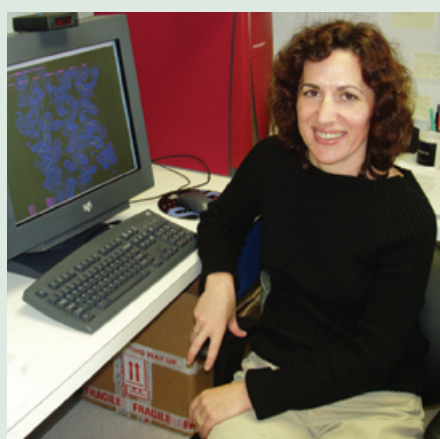


A Salmonella Invasion Protein That Acts as a “Molecular Staple”

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Salmonella invasion protein A (SipA) is an important virulence factor injected into host cells, where it modulates the cytoskeleton by polymerizing actin. By combining high resolution X-ray crystallography of SipA, reconstructions of electron micrographs of actin-SipA filaments, modeling, and structure-based mutagenesis, we demonstrate that SipA functions as a ‘molecular staple,’ in which a central globular domain and two non-globular ‘arms’ mechanically stabilize the filament by tethering actin subunits in opposing strands.



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The etiological agents of plague, typhoid fever, certain food poisonings, and other medically relevant bacterial diseases utilize a type III protein-secretion system to translocate virulence factors, which modulate eukaryotic biochemical processes, into host cells. *Salmonella* species make use of a diverse repertoire of virulence factors, many that target and modulate the structure of the host actin cytoskeleton.

Mutants of *Salmonella typhimurium* with a disruption of the *sipA* gene show an attenuated virulence in bovine intestinal models, impaired ability to invade cells, and less robust and localized membrane ruffling as compared with the wild type strain. Biochemically, the SipA protein of *Salmonella* is able to bind to actin, reduce the critical concentration for the formation of F-actin, stabilize actin filaments, and potentiate the actin nucleating and bundling activity of other virulence factors.

The crystal structure of an active *S. typhimurium* C-terminal domain of SipA (residues 497-669, henceforth SipA⁴⁹⁷⁻⁶⁶⁹) was solved using multiple anomalous dispersion (MAD) techniques on SeMet substituted protein and refined to 1.8Å resolution (**Figure 1**). SipA⁴⁹⁷⁻⁶⁶⁹ possesses a novel three-dimensional structure that, in particular, has no relationship to any known actin binding proteins. This domain of SipA folds into a compact, heart-shaped molecule dominated by helical secondary structure with dimensions of roughly 30x40x40 Å. In the crystal, the protein is ordered only between residues 513 and 669 (leaving 15-20 amino acids disordered at the ends) and the N and C-termini are located at opposite ends of the molecule (the “bottom” and “top,” respectively).

The compact nature of this domain of SipA was unexpected, as previous biophysical and electron microscopic (EM) reconstructions of the larger construct SipA⁴⁴⁶⁻⁶⁸⁴ had indicated that the molecule was quite extended in conformation (~95Å). In order to reconcile these observations, EM studies of G and F-actin in the presence of SipA⁴⁹⁷⁻⁶⁶⁹ were undertaken in collaboration with the group of Edward Egelman at the University of Virginia. These studies reveal that this smaller construct binds to actin as a globular structure with small non-globular extensions (“arms”) that connect different actin monomers (**Figure 1**). Comparisons between EM densities from larger SipA constructs and SipA⁴⁹⁷⁻⁶⁶⁹ reveal that they differ primarily in the length of the non-globular extensions, whereas the central globular density remains similar.

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The fit of SipA⁴⁹⁷⁻⁶⁶⁹ into the EM reconstructions places the SipA termini proximal to the linking arms, suggesting a model in which SipA would function as a kind of “molecular staple,” centered upon the globular domain for binding actin and linking opposite strands using the non-globular extensions. This hypothesis was tested by a series of truncations designed to remove one or both of the “arms” of SipA. Deletion of the arms severely impaired the ability of SipA to polymerize actin, although not its ability to bind pre-formed F-actin, supporting our idea that the arms are key to the linking of actin protomers in the filament.

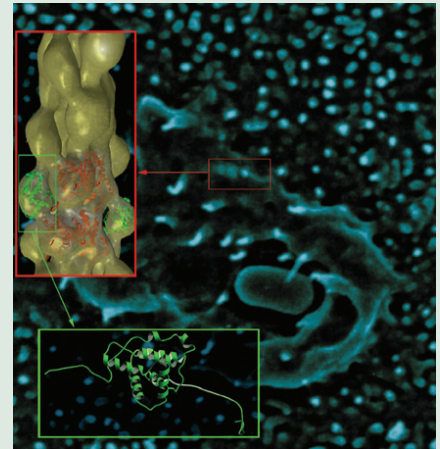


Figure 1. Superimposed on a background of *Salmonella* induced ruffling in intestinal cells is the EM density (red box) of SipA-actin with the actin (red) and SipA (green) crystal structures modeled. In the green box is a model of SipA showing hypothetical “arms” extending from the X-ray structure.